

**IN THE INTERNATIONAL BUREAU OF  
THE WORLD INTELLECTUAL PROPERTY ORGANIZATION**

International Application No. : PCT/US03/27888  
International Filing Date : 05 September 2003 (05.09.03)  
Earliest Priority Date : 06 September 2002 (06.09.02)  
Applicant(s) : ISOTIS SA  
Title : METHODS AND COMPOSITIONS  
FOR TISSUE REGENERATION

The International Bureau of WIPO  
34, chemin des Colombettes  
1211 Geneva 20, Switzerland

**AMENDMENTS UNDER PCT ARTICLE 19**

This amendment of the claims is submitted in response to the International Search Report mailed from the United States Patent Office International Search Authority on 04 December 2003 (04.12.03) for the subject international patent application.

In accordance with PCT Rule 66.8(a), replacement pages 53-58 are attached. In the replacement claims, new claims 1-42 correspond to originally filed claims 1-91. These claims recite a cell preparation for tissue regeneration, kits containing the same, methods of using such kits, and methods of administering the cell preparation of the invention. These amendments were made to more precisely define the claimed invention.

Applicant respectfully requests entry of these amendments in the referenced application under Article 19. No new matter has been added. Substitute sheets of pages 53-58 are hereby submitted.

Favorable action is hereby requested. The authorized officer is invited to contact the undersigned if further information is required.

Respectfully submitted,

  
Christina K. Stock

Jennifer A. Karnakis  
MINTZ, LEVIN, COHN, FERRIS,  
GLOVSKY and POPEO, P.C.

One Financial Center  
Boston, Massachusetts 02111  
United States of America  
Telephone: 617-542-6000  
Facsimile: 617-542-2241

Agent Ref.: 17811-017CIP  
Date: 02 February 2004 (02.02.04)

1. A cell preparation for tissue regeneration comprising
  - a) a first component comprising an extracellular matrix or matrix material containing fibrinogen, wherein the extracellular matrix material is selected from the group consisting of collagens, alginate, alginate beads, agarose, fibrin, fibrin glue, blood plasma, fibrin beads, laminins, proteoglycans, fibronectins, chitosan, and heparin; and
  - b) a second component comprising from about  $1 \times 10^3$  cells/ $\mu$ l to about  $50 \times 10^3$  cells/ $\mu$ l and thrombin to the wound site, wherein the cells comprise one or more keratinocytes and fibroblasts that secrete one or more biologically active molecules selected from the group consisting of GM-CSF, VEGF, KGF, bFGF, TGF $\beta$ , angiopoietin, EGF, IL-1 $\beta$ , IL-6, IL-8, TGF $\alpha$ , and TNF $\alpha$ , and wherein the one or more keratinocytes and fibroblasts are allogeneic and mitotically active or inactivated.
2. The cell preparation of claim 1, wherein the one or more keratinocytes and fibroblasts are differentiated fibroblasts and keratinocytes.
3. The cell preparation of claim 1, wherein the cell preparation is in the form of a paste.
4. The cell preparation of claim 1, wherein the cell preparation is in the form of a spray.
5. The cell preparation of claim 1, wherein the one or more keratinocytes and fibroblasts are mitotically inactivated by administration of mitomycin C or other chemically-based mitotic inhibitors, irradiation with  $\gamma$ -Rays, irradiation with X-Rays, or irradiation with UV light.
6. The cell preparation of claim 1, wherein the one or more keratinocytes and fibroblasts are immortalized using at least one gene/polypeptide selected from the group consisting of the 12S and 13S products of the adenovirus *E1A* genes,

hTERT, SV40 small T antigen, SV40 large T antigen, papilloma viruses E6 and E7, the Epstein-Barr Virus (EBV), Epstein-Barr nuclear antigen-2 (EBNA2), human T-cell leukemia virus-1 (HTLV-1), HTLV-1 tax, *Herpesvirus saimiri* (HVS), mutant p53, *myc*, *c-jun*, *c-ras*, *c-Ha-ras*, *h-ras*, *v-src*, *c-fgr*, *myb*, *c-myc*, *n-myc*, and *Mdm2*.

5

7. The cell preparation of claim 1, wherein the one or more keratinocytes and fibroblasts naturally secrete one or more biologically active molecules.

10 8. The cell preparation of claim 1, wherein the one or more keratinocytes and fibroblasts are genetically engineered to secrete an exogenous level of one or more biologically active molecules.

15 9. The cell preparation of claim 1, wherein the secretion of the one or more biologically active molecules is controlled by gene switching.

10. The cell preparation of claim 1, wherein the one or more biologically active molecules is constitutively secreted.

20 11. The cell preparation of claim 1, wherein the first component comprises fibrinogen.

12. The cell preparation of claim 1, wherein the second component optionally further comprises a cryoprotectant.

25

13. The cell preparation of claim 12, wherein the cryoprotectant is selected from the group consisting of a 10% glycerol solution, a 15% glycerol solution, and a 15% glycerol and 5% human serum albumin solution

30 14. The cell preparation of claim 13, wherein the cryoprotectant is glycerol.

15. A kit comprising, in one or more containers, the cell preparation of claim 1.
- 5 16. The kit of claim 15, wherein the first component and the second component are cryopreserved prior to shipping and subsequently thawed prior to use.
- 10 17. The kit of claim 16, wherein the first and second components are each contained in a separate vial having a removable screw cap, wherein the vial is sterile and is made of a material resistant to low temperatures and wherein the removable lid can be replaced with a spray pump following thawing of the first and second components prior to use.
- 15 18. The kit of claim 17, wherein the spray pump delivers a volume of approximately 130  $\mu$ l per spray.
19. The kit of claim 17, wherein the material resistant to low temperatures is selected from the group consisting of glass, polypropylene, polyethylene, and ethylene vinyl acetate (EVA).
- 20 20. The kit of claim 17, wherein the vials are sealed within a pouch or container prior to cryopreservation, wherein the pouch or container is fabricated of a material capable of withstanding temperatures ranging from -80°C to -196°C and wherein the pouch or container protects the first and second components from contamination during cryopreservation and subsequent thawing.
- 25 21. The kit of claim 20, wherein the pouch or container is waterproof and has a high barrier performance.
- 30 22. A method of using the kit of claim 15, the method comprising  
a) administering the first component to a wound site on a patient in need of treatment; and

- c) combining the first and second components to form the cell preparation;  
and
- d) administering the cell preparation to the wound site.

5     32.     The method of claim 31, wherein the first and second components are topically applied to the wound site

33.     The method of claim 31, wherein the first component is applied to the wound site before the second component is applied to the wound site.

10

34.     The method of claim 31, wherein the second component is applied to the wound site before the first component is applied to the wound site.

35.     The method of claim 31, wherein the cell preparation is in the form of a paste.

15

36.     The method of claim 31, wherein the cell preparation is in the form of a spray.

37.     The method of claim 31, wherein the second component optionally further comprises a cryoprotectant.

20

38.     The method of claim 37, wherein the cryoprotectant is selected from the group consisting of a 10% glycerol solution, a 15% glycerol solution, and a 15% glycerol and 5% human serum albumin solution

25     39.     The method of claim 31, wherein the first and second components are sprayed on the wound site.

40.     The method of claim 39, wherein the first component is sprayed on the wound site before the second component is sprayed on the wound site.

30

- b) combining the second component with the first component wherein the combination of the first component and the second component forms a cell preparation suitable for tissue regeneration.

5    23.    The method of claim 22, wherein the cell preparation is in the form of a paste.

24.    The method of claim 22, wherein the cell preparation is in the form of a spray.

10    25.    The method of claim 22, wherein the second component optionally further comprises a cryoprotectant.

26.    The method of claim 25, wherein the cryoprotectant is selected from the group consisting of a 10% glycerol solution, a 15% glycerol solution, and a 15% glycerol and 5% human serum albumin solution

15    27.    The method of claim 22, wherein the first and second components are topically administered to the wound site on the patient.

20    28.    The method of claim 22, wherein the first and second components are sprayed onto the wound site on the patient.

29.    The method of claim 28, wherein the first and second components are combined on the wound site.

25    30.    The method of claim 28, wherein the first and second components are combined before reaching the wound site.

31.    A method of administering cell preparation of claim 1 to a wound site on a patient in need of treatment, the method comprising

- 30    a)    providing the first component;
- b)    providing the second component;

41. The method of claim 39, wherein the sprayed first and second components are combined on the wound site.
42. The method of claim 39, wherein the sprayed first and second components are combined before reaching the wound site.
- 5

TRA 1869990v1